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Key role of sinusoidal endothelial cells in the triggering of liver regeneration

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COMMENTARY ON:

Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. Ding BS, Nolan DJ, Butler JM, James D, Babazadeh AO, Rosenwaks Z, Mittal V, Kobayashi H, Shido K, Lyden D, Sato TN, Rabbany SY, Rafii S. Nature. 2010 Nov 11;468(7321):310–5. Copyright (2010). Abstract reprinted with permission from Macmillan Publishers Ltd.: [Nature].

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Abstract: *During embryogenesis, endothelial cells induce organogenesis before the development of circulation. These findings suggest that endothelial cells not only form passive conduits to deliver nutrients and oxygen, but also establish an instructive vascular niche, which through elaboration of paracrine trophogens stimulates organ regeneration, in a manner similar to endothelial-cell-derived angiocrine factors that support hematopoiesis. However, the precise mechanism by which tissue-specific subsets of endothelial cells promote organogenesis in adults is unknown. Here we demonstrate that liver sinusoidal endothelial cells (LSECs) constitute a unique population of phenotypically and functionally defined VEGFR3(+)CD34(−)VEGFR2(+)VE-cadherin(+)FactorVIII(+)CD45(−) endothelial cells, which through the release of angiocrine trophogens initiate and sustain liver regeneration induced by 70% partial hepatectomy. After partial hepatectomy, residual liver vasculature remains intact without experiencing hypoxia or structural damage, which allows the study of physiological liver regeneration. Using this model, we show that inducible genetic ablation of vascular endothelial growth factor (VEGF)-A receptor-2 (VEGFR2) in the LSECs impairs the initial burst of hepatocyte proliferation (days 1–3 after partial hepatectomy) and subsequent reconstitution of the hepatovascular mass (days 4–8 after partial hepatectomy) by inhibiting upregulation of the endothelial-cell-specific transcription factor Id1. Accordingly, Id1-deficient mice also manifest defects throughout liver regeneration, owing to diminished expression of LSEC-derived angiocrine factors, including hepatocyte growth factor (HGF) and Wnt2. Notably, in in vitro co-cultures, VEGFR2-Id1 activation in LSECs stimulates hepatocyte proliferation. Indeed, intrasplenic transplantation of Id1(+/+) or Id1(−/−) LSECs transduced with Wnt2 and HGF (Id1(−/−)Wnt2(+)HGF(+) LSECs) re-establishes an inductive vascular niche in the liver sinusoids of the Id1(−/−) mice, initiating and restoring hepatovascular regeneration. Therefore, in the early phases of physiological liver regeneration, VEGFR2-Id1-mediated inductive angiogenesis in LSECs through release of angiocrine factors Wnt2 and HGF provokes hepatic proliferation. Subsequently, VEGFR2-Id1-dependent proliferative angiogenesis reconstitutes liver mass. Therapeutic co-transplantation of inductive VEGFR2(+)Id1(+)Wnt2(+)HGF(+) LSECs with hepatocytes provides an effective strategy to achieve durable liver regeneration.*

Although liver regeneration has been known since antiquity and was already clearly described by Higgins and Anderson [1], the precise molecular and cellular mechanisms driving the regenerative capacity of the liver are still not fully comprehended. Liver regeneration after partial hepatectomy is characterized by a compensatory hyperplastic response of the mature differentiated remnant liver cells, which lasts until the original liver mass is restored. The transition from quiescent to proliferative hepatocytes is regulated by paracrine factors such as cytokines and growth factors in a chain reaction involving different liver cell types. The priming phase (G0/G1 transition) is achieved by macrophage activation via the complement cascade components C3a [2] and C5a [2] and the bacterial endotoxin lipopolysaccharide (LPS) [3], inducing secretion of TNFα [4] and IL6 [5] cytokines. The IL6/gp130-dependent pathways have been presumed to trigger hepatocyte proliferation via the activation of the JAK/STAT [6] and MAPK [7] pathways. However, liver DNA synthesis post-hepatectomy was abundant in gp130-deleted mice, indicating that the key players of the early phases of liver regeneration were still to be identified [8].

The groundbreaking work of Ding *et al.* recently published in *Nature* reveals that the liver sinusoidal endothelial cells (LSECs), which harbor a specific $VEGFR2^{+}$ $VEGFR3^{+}$ $CD34^{-}$ VE^{-} $cadherin^{+}$ $FactorVIII^{+}$ $CD45^{-}$ phenotype play a crucial role in the triggering of hepatocyte proliferation [9]. These authors report a biphasic proliferative wave of hepatocytes during the first 3 days after partial hepatectomy, and then of LSECs from day 4 to day 8. Using knockout mice models, they showed that partial hepatectomy induced VEGFR2 activation at cell surfaces of LSECs, initiating

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Id1 up-regulation and secretion of HGF and Wnt2 angiocrine factors. The production of HGF and Wnt2, as well as the direct contact between LSECs and hepatocytes were clearly necessary conditions for the first wave of hepatocytes proliferation to occur. Subsequently, the VEGFR2-Id1 pathway promoted neoangiogenesis to ensure blood supply of the growing liver. Ding *et al.* raise the unsolved question of how LSECs sense partial hepatectomy and suggest that they respond to some imbalance of the inhibitory factors, which maintain the mass of the liver. These conclusions may be compared to those of a recent work by Ninomiya *et al.* showing that a decrease in liver regenerative speed caused by ERK/MEK inhibitors reduced the small-for-size syndrome in 70% or 90% partial hepatectomy in rats [10]. The latter authors conclude that the abrupt regenerative response of hepatocytes to resection stifles the sinusoids, resulting in hypoxia of the hepatocytes and liver dysfunction. A synchronized replication of hepatocytes and SECs is thus a crucial requirement for proper liver regeneration.

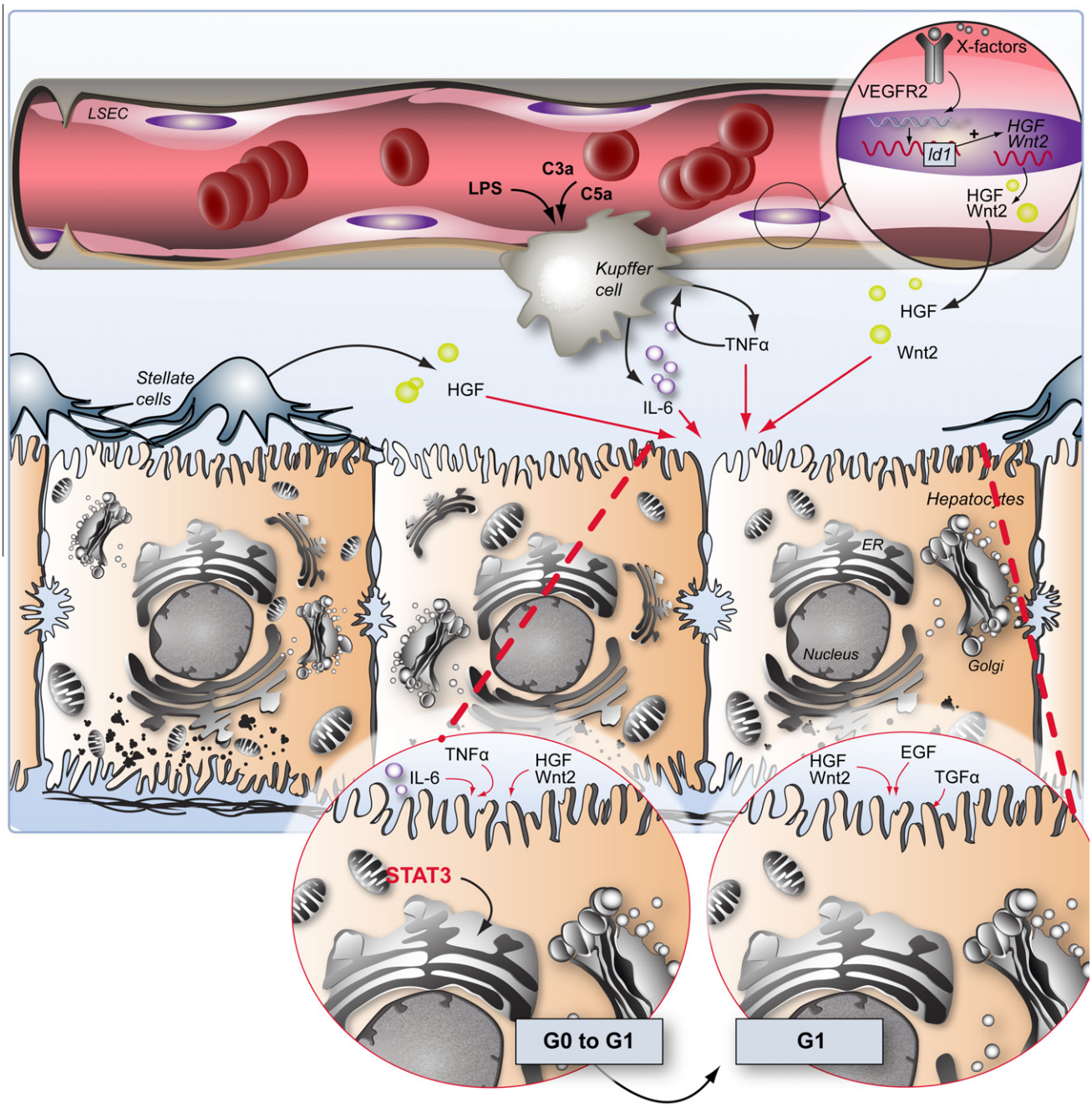

Fig. 1. Schematic representation of the priming events leading to liver reconstruction after partial hepatectomy. Hepatocytes priming is induced by the cumulative action of TNFα and IL6 secreted by activated Kupffer cells, HGF secreted by activated stellate cells, and HGF and Wnt2 secreted by LSECs. Unknown soluble factors activate LSECs via VEGFR2, leading to HG and Wnt2 expression and secretion via Id1 up-regulation.

Hence, recent research findings point toward the importance of SECs in the hepatocyte response to partial hepatectomy and should impact the design of future regenerative medicine based on hepatocyte or stem cell transplantation for the treatment of end-stage liver diseases. Worldwide, there is a growing interest for such innovative cell-based therapies as an alternative for orthotopic liver transplantation. If numerous clinical reports have established the efficacy of cell infusions therapies for the correction of inborn metabolism disorders, their clinical potential for the treatment of acute liver failure remains uncertain, mostly because engraftment efficiency can only be poor in a situation without proper liver architecture. In the light of the current results, improvement of these therapies may require the co-infusion of hepatocytes/stem cells with SECs or the use of a mimetic scaffold. In addition, Uygun *et al.* demonstrated that hepatocytes engrafted into a decellularized liver scaffold, allowing a perfect lining of hepatocytes along fully functional SECs, was much more efficient than direct transplantation within the liver [11].

In conclusion, Ding *et al.* have cast light on several crucial aspects of the regulation of the initial steps of liver regeneration and DNA synthesis in hepatocytes. Using VEGFR2 and Id1 knockout mice enabled them to show that HGF production by non-endothelial cells, such as stellate cells, was not a sufficient condition for hepatocyte proliferation. Secretion of HGF and Wnt2 by LSECs was also necessary (Fig. 1). The identification and characterization of the soluble factors that promote activation of LSECs and HGF/Wnt2 secretion will provide valuable tools for future therapeutic developments. In agreement with their conclusion that close contact between LSECs and hepatocytes was necessary for triggering liver regeneration, Ding et al. suggest to improve on the current methods of cellular transplantation by co-transplanting LSECs with hepatocytes in patients with liver insufficiency. This is an interesting concept, which deserves to be tested in models of damaged liver to assess the role of LSECs in the regeneration of livers subjected to multiple stress factors.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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